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A METHOD OF DETERMINING IN ANALYTIC WORK WHETHER COLONIES OF THE CHESTNUT BLIGHT FUNGUS ORIGINATE FROM PYCNOSPORES OR ASCOSPORES[1]

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(With Plates 98–101)

Introduction

In studying the dissemination of the chestnut-tree blight fungus [*Endothia parasitica* (Murr.) And. and And.] it is sometimes of importance to be able to determine whether the colonies of *Endothia* appearing in poured plates originate from pycnospores or ascospores. This is especially true in case of the analysis of soil for the presence of the fungus, the quantitative determination of viable spores retained in spore traps, and other similar operations.

During the past winter thousands of cultures of the blight fungus have been made, especially in studying the problem of dissemination, but before beginning the work the method here outlined was worked out, as it appeared to the writer of fundamental importance.

At first thought the possibility of differentiating ascospore and pycnospore colonies seemed somewhat remote, but the striking difference in size of pycnospores and ascospores offered the first clue to the problem. The approximate dimension of the spores (ascospores $5 \times 10\,\mu$; pycnospores $1 \times 3\text{–}5\,\mu$) gives rather an imperfect notion of their difference in magnitude, but calculation will show that the ascospore of average size has a volume about fifty times that of the average pycnospore. It seemed evident then that the greater size of the ascospore would result in a more rapid growth of the colonies originating from them.

[1] Work in coöperation with the Pennsylvania Chestnut-Tree Blight Commission, Philadelphia, Pa.

The medium found most suitable for this work was 3 per cent. dextrose agar, plus 10, made according to the standard bacteriological formula. The comparative rate of growth from ascospores and pycnospores was first tested in this medium by means of hanging-block cultures. The pycnospores used were obtained from spore-horns grown in damp chambers in the laboratory. The ascospores were obtained by placing flamed object slides over moistened bark bearing perithecial pustules and collecting the expelled spores. In making the pycnospore cultures a drop of sterile bouillon was placed on a flamed slide and a small spore-horn added to it. One or more dilutions were made from this to other drops of sterile bouillon and a short streak was made from the final dilution upon the surface of the cover glass, after which the streak was covered with melted agar cooled to 42° C. In making the ascospore cultures a drop of sterile bouillon was placed over a spore print on a slide. Dilutions were made from this to a second slide, and the planting made directly from the spore dilution.

By these methods there was never any trouble in securing pure cultures in the hanging drop cells.

GERMINATION OF PYCNOSPORES AND ASCOSPORES

During the first part of the germination period the pycnospore increases in size until it is oval or oblong in form and slightly in excess of the diameter of the germ tube that is to be produced (plate 98, figs. 1–3). A hypha begins to grow out from one end of the spore and this is generally followed later by one from the opposite end so that at temperatures from 22° to 25° C., only an unbranched linear aggregate has been produced at the end of 24 hours. During the next 24 hours, however, branching generally begins, the first branch originating a little beyond the limits of the spore, thus producing a distinct Y-type of growth (plate 98, figs. 4–6).

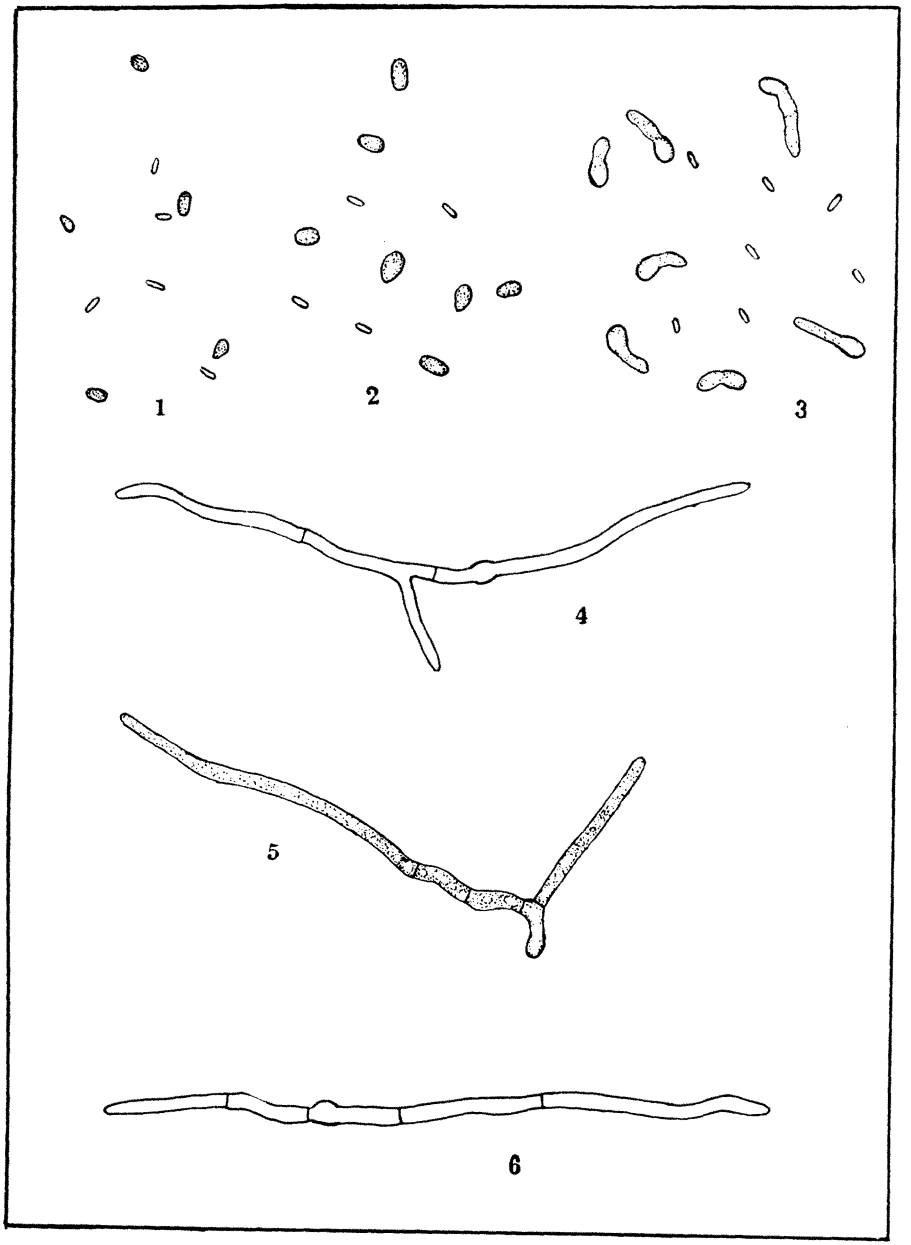
Each cell of an ascospore generally gives rise directly to at least one vigorous hypha, but occasionally one cell fails to germinate. In many spores each cell gives rise to a lateral hypha a little later. In case a lateral hypha is not formed directly from

the spore cell, one originates a few mikrons beyond the spore wall, giving in the majority of cases a growth with pronounced decussating branches (plate 99, figs. 1–5).

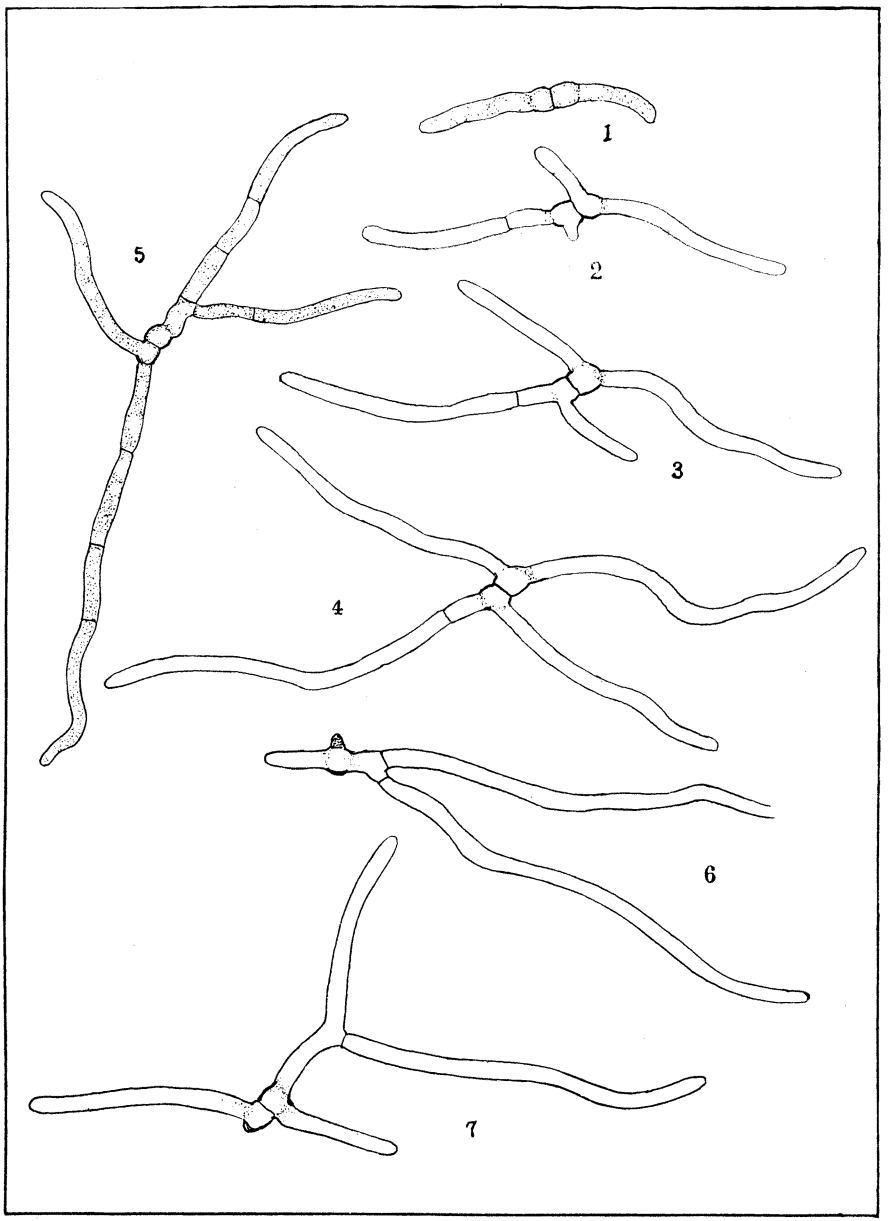
Tests of the comparative rate of growth of pycnospores and ascospores were first made at 22° C., but it was found by later work that 25° C. gave a more pronounced difference. Camera lucida drawings were made at hourly intervals for the ascospores after the first eight hours and at intervals of two hours for the pycnospores. At the end of eight hours the ascospores produced a strong hypha from each cell, while the pycnospore had not yet swollen to its full size; after 22 hours the pycnospore had produced a short unbranched hypha with little or no septation, while the ascospore had produced a much branched linear aggregate of cells (plate 100). The series of drawings shown will serve to emphasize the pronounced difference in the growth from the pycnospores and ascospores.

PLATE CULTURES

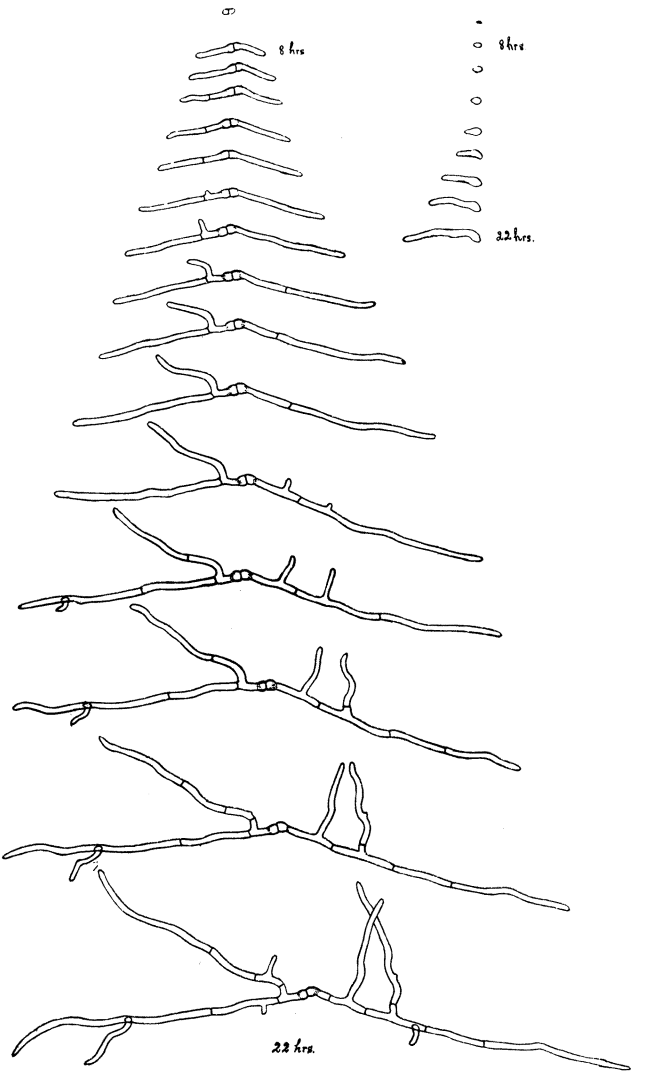
The marked difference in the rate of growth from pycnospores and ascospores suggested the strong probability of being able to differentiate the two types of colonies in plate cultures by the size of the colonies at the end of a certain time. Various culture media were tried but 3 per cent. dextrose agar, plus 10, again appeared to be the most suitable. The poured plates were made in the usual manner from spores obtained in the same way as for the hanging-block cultures, and practically pure cultures of the blight fungus were always obtained. The cultures were held at a constant temperature of 25° C. All of the tests made showed that ascospore colonies became visible and conspicuous when the pycnospore colonies were still minute and invisible to the naked eye. At the end of three days' time, colonies originating from ascospores were 0.5–3 mm. in diameter, the size depending upon the crowding in the plate, while those originating from pycnospores were not visible to the naked eye (plate 101); after four days of growth the ascospore colonies were 1–4 mm. in diameter while the pycnospore colonies showed an average diameter of 400 μ. The time of appearance of the yellow centers in the colo-



GERMINATION OF PYCNOSPORES



GERMINATION OF ASCOSPORES



SERIES SHOWING COMPARATIVE GROWTH IN HANGING DROP CULTURES

COMPARATIVE GROWTH FROM ASCOSPORES AND PYCNOSPORES

nies does not appear to be of importance since this varies according to crowding, depth of medium and origin. The reliability of this method for differentiating pycnospore and ascospore colonies has been substantiated by numerous cultures, but the importance of holding the cultures at a constant temperature must not be overlooked.

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EXPLANATION OF PLATES

PLATE XCVIII

Germination of pycnospores in 3 per cent. dextrose agar, plus 10, at 22° C: 1, after 12 hours; 2, after 16 hours; 3, after 22 hours; 4, 5, 6, after 36 hours. These illustrate the linear and the Y-types of germination.

PLATE XCIX

Germination of ascospores in 3 per cent. dextrose agar, plus 10, at 22° C. 1–4, a series showing stages in the growth from a single spore: 1, at 11:45 A.M.; 2, at 2:45 P.M.; 3, at 4:45 P.M.; 4, at 7:45 P.M.; 5, 6, 7, after 24 hours. In 4 each cell has produced two hyphae; in 5 one cell has produced two hyphae, while a strong lateral has grown out from the main axis just beyond the other cell of the spore; in 6 one cell has produced a short lateral but no terminal hypha; in 7 one cell of the ascospore failed to produce a germ tube.

PLATE C

A series of drawings showing the comparative growth from an ascospore and a pycnospore in 3 per cent. dextrose agar, plus 10, at a temperature of 25° C. After eight hours drawings were made at hourly intervals for the ascospore series and every two hours for the pycnospore series.

PLATE CI

Poured plate cultures of ascospores and pycnospores to show comparative growth in 3 per cent. dextrose agar, plus 10, at a temperature of 25° C. Photograph taken after three days of growth. Pycnospore colonies not yet visible to the naked eye.